

December 25, 1952

Dear Luca:

You do right to reproach me for my silence, but it is mainly that I have had very little to say. I am glad that the hectic problems of the JGM ms. will finally be resolved (may we hope); there was very little that I could do to help, and I had full confidence in your judgment. It is just a multitude of other duties and details that have kept me from being more communicative. At any event, though, I would like to wish you the very best for the new year-- on my part this would include the possibility of some more personal contacts. Frankly, I have been very much distracted for the past two months with Salmonella problems, although they may in the long run shed some light on recombination in *E. coli* (at least in a negative way). I will mention some more of this below.

I am not surprised you could find no linkage with F^+ , if its segregation behavior is already unique in giving independent segregation. As I may have mentioned, I was working earlier on the segregation of Hfr in crosses with F^- , but found nothing very definite except that a nondisjunctional type, H-310 (from M-Hfr x W1177, on Δ MB Lac sm) behaved as if it was itself Hfr in crosses. H-310 was picked as S^+ Mal- V_1^+ Xyl- TLB₁- but Lac+/-, which is already very strange. None of the pure Lac+ or Lac- segregants that I have tested have been anything but F^- . H-310 does behave as a diploid, however, for cross H-310 x W-1895 have given occasional "complete" diploids, e.g. H-313 which is segregating at S, Mal, Mtl, H, TL, though pure Lac+ (not surprising). I was very pleased at this. The experiments were designed to get exceptional diploids which might be Mal+ F^- /Mal- Hfr, to check the speculation that the Hfr locus might be linked to Mal, and in the usual course of events be eliminated with Mal, accounting for getting only F^- from Hfr x F^- . I did not realize then that H-310 was Hfr, as I had tested only the segregants. H-313 turned out to give segregants (5 tested) all Hfr, whether Mal+ or Mal-. This led me to test H310. I am still speculating that H310 is a nondisjunctional of the general structure: M- Lac+ Mal- S^+ F^- / M+ Lac+ Mal- S^+ Hfr, but that in this case ~~Recombinationally, nondisjunctional, nondisjunctional, nondisjunctional, nondisjunctional~~ as suggested for others, Hfr is eliminated during segregation. H313, then would be homozygous Hfr, having been derived from the strand "b" of H310 + W-1895. Thus, it has been possible to secure Hfr in combination with other markers, i.e., TLB₁- or Lac- etc.) One of these segregants, W-2060, TLB₁- Mal+ S^+ V_1^+ gave an unusual result in a single test: I was actually looking for an F^- lambda prototroph recombinant to use as a starting culture for further mutant isolations, and therefore crossed W-2060 x W-1578 (P-G- λ lambda^S). Only one prototroph was examined, but it was Hfr! This would be the first time that Hfr x F^- , in a haploid cross, given Hfr. It will have to be studied further. Any of these stocks are, of course, available to you. The notion of a Mal-F linkage in the eliminated segment could be strengthened if I could get a Mal+/Mal- reversion from H-310, but for some reason I have not been able to do this. Other experiments to try to secure exceptional "complete" diploids, usually by crossing previous Mal+ exceptions (viz. figure 4E, ~~22x~~ CSH '51 paper) to M-Hfr gave unintelligible results, the analysis of the diploids being too difficult for purely technical reasons. In fact, I did not have the best material on hand for these experiments, and it would be better to use the Hfr segregants from H313 and cross these to F^+ diploids. The peculiar result with W-2060 is, however, already a lead in another direction. If I had some F^- diploids, it would be ideal, but these are almost impossible to obtain (by definition).

As I may already have mentioned too many times, I have permitted myself to be distracted away from cytological studies on Hfr for the time being, and have rationalized this by waiting for Tom Nelson to get somewhere with his kinetic and physiological studies. It has taken some time

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by the interesting possibilities of polygenic analysis of resistance to nitrofuracin, which turned up incidentally to looking for additional technical tools for studying recombination. His results may be summarized as follows (most experiments with W-1895 (M-Hfr) x W-1956 (=W1177 V₆^R).

A: crosses in broth, count Lac⁺ S^r as recombinants on EMB Lac SM (this is easier and equivalent to counting Lac⁺/- segregating colonies on EMB Lac).

rapid, but not

With fairly dense inocula, there is about 15 minutes lag before/exponential growth. Recombinants increase linearly, more rapidly than growth, but also after a 15 minute lag. They level off at about 5% of the total population. The lag is not eliminated by starting growth of separate cultures first, suggesting some sort of ~~mutual~~ mutual conditioning.

Adding new medium permits increase in absolute numbers of "zygotes", but not their proportion. Adding more cells of either parent alone does not increase zygotes, suggesting that it is the medium that has become unfavorable, rather than one class of cells that is exhausted. The "collision efficiency", i.e., ratio of zygotes to estimated number of collisions, calculated from the linear increase of zygotes, is approximately 1%. This seems to make it necessary to assume that every bacterium is competent, but makes it difficult to understand why the limiting ratio of zygotes to bacteria should be only 5%. (We are ignoring possibility of segregation of zygotes during the experiments). Aeration of the Hfr parent reduces rate and extent of zygote formation (about 100fold as measured by prototrophs).

A few experiments have been done to define the physiological necessities for Hfr. Cells were concentrated from broth, and made up to high densities in broth: this prevented Hfr, as detected by Lac⁺S^r (let me call this method "SR⁺"). In crosses ~~W-1895 (M-Hfr) x furacin resistant (W-1895 Nf^r)~~ x W-1956, the zygotes appeared to be sensitive to Nf^r. W-1895 x W1956Nf^r, as well as W-1895 Nf^r x W1956, the zygotes appear to be sensitive to the furacin (unlike the sm anomaly of Hayes). This needs further study, however, as there is some zygote-inhibition by furacin even with Nf^r x Nf^r. [I just realize the unsuitability of Nf^r as a symbol; let me abandon it in favor of Fx^r]. Tom has timed segregation in the cross 1895 x 1956Fx^r, where most of the segregants are Fx^r, where it appears to begin at about 30 minutes. This is consistent with casual observations on the incidence of pure vs. sectored SR⁺.

Kinetic studies are very difficult under growing conditions. Tom has spent a lot of time on the kinetics of recombination in buffer, although the rates here are only about 1% as high as in broth, calculated on the basis of collision efficiency. Quite good fits to a bimolecular reaction were obtained, except for a levelling off at a zygote:bacteria ratio of about .2%. The rate constants for the linear portions were about 10⁻¹³/min. The highest rates in his earlier expts (F⁺ x F⁻) were about 10⁻¹⁶. ~~From about 10⁻¹³ to 10⁻¹⁶ min⁻¹ depending on the amount of broth added.~~ It is still possible that crosses in buffer reflect a purely physical process (clumping?), and that the interesting events occur on the agar plates. There is some substantiation of this (in prototroph expts) in that the slope of zygotes as a function of time of contact is increased by adding small amounts of broth, in addition to the trivial increase in plate recombinants. One may add to this that the absolute number of recombinants, either SR⁺ or prot., decreases to 10-30% of the optimal number if the crosses are held a long time in broth, buffer or saline. All this suggests there is a stage of zygote formation which is more sensitive to physiological conditions than the preliminary stages or than growth. A further indication of this is that recombination is accelerated severalfold by adding small amounts of broth to buffer, which have only a very small effect on increasing the cell count.

For planning cytological experiments, obviously the most important thing is to get optimal rates of zygote formation with minimum growth. It is already clear that I had not been achieving the optimal conditions in my earlier cytological preliminaries (saturated cell populations etc). So far, recombination without growth has not been feasible. Low temperatures inhibit both. However, more work remains to

be done with synthetic media.

I have not yet gotten Tom to do much on the kinetics of F+ transduction; some of his preliminary experiments were disappointing (not necessarily meaningfully so), but I hope he will accelerate along these lines. We are talking especially of verifying that phenotypically F-, aerated 58-161 cells are still infective. I suppose W.H. would interpret ~~this~~ this as meaning that aeration simply prevents the bacterial nucleus from entering the infective gamete. If as I suspect, the agent of recombination is the whole cell, and the process involves the transmission of a single nucleus following a brief conjugation, there will be a very close formal similarity between the hologamete and WH's infective gamete. One can argue that in Hfr crosses, every infective gamete carries a nucleus, but this still would not explain the ~~exclusive~~ exclusive production of F- from most Hfr x F- crosses.

Just a word on Salmonella:

Transduction is certainly mediated by phage particles. An individual particle has a probability of ca 10^{-6} of carrying a particular trait from the host on which it has just been grown. The FA particles agree with phage in filtration, adsorption on bacteria, inactivation by antiserum. The incidence ~~of~~ induced lysogenicity is higher in transduced bacteria than in the rest. Recently, I have found a lytic mutant (22V) with the interesting property that it will not lyse bacteria that have adsorbed the temperate phage or are lysogenic for it. This should permit a conclusive proof that transduced bacteria have ~~been~~ adsorbed phage, which will be especially meaningful when the phage:bacterium ratio is small. UV will inactivate lytic power of phage while hardly affecting FA. X-rays are almost ineffective at reasonable doses (10^5 r).

Numerous serotypic recombinants have been made. With diphasic species, each phase is transduced separately. Judging from the difference in activity of FA from the two phases of *S. typhimurium*, phase variation is due to a "change of ~~state~~ state" reciprocally between two loci. Unlike *Paramecium*, this change is at the locus (accompanying it during transduction) rather than in the cell as a whole. I must have mentioned Stocker's experiments to you in previous letters. He is writing them up now. One case of linkage: a motility factor (Fla_1) and the specific antigen (H_1^i), as shown by getting both H_1^b and H_1^i in selections for motility in $Fla_1^+ H_1^i \rightarrow Fla_1^- H_1^b$. [\rightarrow means transduction to]. Fla and H may conceivably be "pseudoalleles".

Have I mentioned a transduction in K-12? Lambda will transduce Gal_4^+ , to which it is very closely linked. It will also transduce other members of a complex of Gal factors whose allelisms have not been entirely disentangled, but not any other factors. Transduction occurs unhindered in F- \rightarrow F- combinations, and, of course, recombination does not require lambda, so these are still entirely separate phenomena. The evidence that lambda is the transducing agent is fairly complete. A difference from Salmonella is that many of the transductions are unstable, the Gal^+ selections from $Gal^+ \rightarrow Gal^-$ tending to become Gal^- again, and as already mentioned, other factors besides Gal have not been transduced. We would prefer not to make public references to these findings (in print, that is; it is no secret, and most of the American phage workers have heard it) until they have been cleared up. It should not be necessary to alter statements already in press, although it may be advisable in future to be cautious about denials that any markers can be transduced in *E. coli*. I could have sworn that I had sent you details on this work which has been done the last 2 or 3 months by M. L. Morse and my wife, but I admit I cannot find the evidence of it in the carbon copies of my letters. If so, I ~~am~~ am most apologetic. Our correspondence was so much about MSS that it must have been crowded out of my head.

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My associate P.D. Skaar did some crude experiments with "F+" antiserum, but nothing worth mentioning (positive or negative) came from it. We should try antiserum to inhibit recombination; agglutination with K-12 is almost meaningless. Would the following suit you: if you can get any qualitative effects, Tom will do the rather laborious rate measurements. Skaar did not succeed in showing any difference between anti-F- and antiF+ with adsorptions, but as I said, these were rather crude. What line of E. coli does your "foreign F agent" refer to? It is most interesting if foreign agents will permit $F^+ \times F^+$. Is it possible that the K-12 agent will behave similarly, but may be unstable also? This could be checked using W-1305 as the F+ donor.

Concerning Italian personalities, I will write to Zironi asking for a reprint of his paper (1938 Boll. Soc. Int. Sez. Ital), if he still has same— if it would be appropriate for you to relay my request as well, I would appreciate it. In fact, our library should have a complete set of these proceedings, but has only 1930-31-32. Do you know if the others are still on sale, and at what price? I notice they were published by your Istituto. If you could get me photocopies (microfilm will be fine) of Denes (G. Batt. Imm. 15:60, '35); ~~xxxx~~ Bizzarri (1937 Boll Soc.... 9:260) and Muremtsev (Ann. Igiene 3:379) I would appreciate it. I am reviewing paraggglutination, which is as confused a subject, historically, as could be so that even inconclusive papers may give me useful clues. If I had not mentioned it before, Dianzini has moved to the University of Genova, Department of General Pathology & Bacteriology, the University.

I hope this clears up all the loose ends; let me know if not.

And, with best wishes again for 1953,

Sincerely,


Joshua Lederberg